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We studied healthy recruits (controls), and recruits with exertional heat illness (EHI) during basic training. We copied pertinent clinical and training records on all identified EHI cases during this reporting period, numbering approximately 100 cases--fewer than usual, but the 1997 hot season was cooler than usual until mid-August. Unlike past years, when most EHI cases occurred during the first 4 weeks of training, and while running, 40% of EHI cases occurred during a 52-h exercise, the "Crucible" in the 11th (next-to-last) week of training. The Crucible, new this year, involves no significant running, but includes two 9-mile marches with pack, with no more than 4 hours sleep a night. In 20 healthy recruits, we measured core temperature (T_{core}) with swallowed telemetering sensors from 0500 to 1700 hours each day of one Crucible, and collected 7 blood samples at 12-h intervals, starting the day before. In 80 other recruits in four cohorts, we measured T_{core} during middle-distance (2.5 to 4 mile) runs at three stages of training, and the final march of the Crucible. We also obtained blood samples on enrollment and initial, final, and recovery sample with each event, for selected clinical measurements and assessments of immune/inflammatory function.

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C. Bruce [illegible] 29 October 1997

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INTRODUCTION

In this section, material of a general nature that is not specific to this reporting period but is included for the sake of completeness and to allow this progress report to stand on its own, is italicized.

An epidemiological study of exertional heat injury (EHI) in recruits undergoing basic training at Parris Island Marine Corps Recruit Depot (MCRD) has shown interesting differences between male and female recruits in the clinical presentation of EHI. Although incidence of EHI is similar in male and female recruits, 11% of male EHI patients, but <1% of female EHI patients are hospitalized. In non-hospitalized EHI patients, presenting rectal temperature is higher in the males, yet serum levels of enzymes indicating tissue injury are higher in the females (Table 1). These differences suggest that there may be differences between men and women in the pathogenesis of, and risk factors for, EHI.

In this recruit population, individual risk of EHI is strongly associated with time to complete the 1½-mile run in the first physical fitness test (PFT) during recruit training, and with body mass index (BMI) (weight/height^2) which correlates with obesity. In a recently published series (Gardner, Kark, Karnei, Sanborn, Gastaldo, Burr, and Wenger, 1996), recruits in the slowest quartile by run time had a 3-fold increase ($p<10^{-5}$) in risk of EHI compared with recruits in the fastest quartile. Male recruits in the highest quartile for BMI had more than a 3-fold increase ($p<10^{-5}$) in risk of EHI compared with recruits in the

lowest quartile for BMI, and male recruits with both high BMI and long run times were at more than 6 times the risk of EHI as those who were thin and fast. Thus in men, at least, PFT run times and BMI are virtually independent predictors of risk of EHI. (Among female recruits, however, individuals in both the highest and the lowest quartiles for BMI were at higher risk of EHI than those in the middle two quartiles.) Because of these relationships, it is a relatively simple matter to define and study subpopulations with substantially different individual risks of EHI.

The ratio of an individual's maximal rate of O_2 uptake (Vo_2max) to body weight (this ratio is sometimes called "relative Vo_2max) is an excellent predictor of his or her effectiveness in limiting increases in body core temperature (T_{core}) during exercise-heat stress (Davies, Brotherhood, and Zeidifard, 1976), and there is a strong inverse correlation between relative Vo_2max and best run time over a given distance. Therefore one should expect PFT run time to be a good predictor of the risk of EHI. Because of the relation of BMI to obesity, and the relation between obesity and relative Vo_2max (Buskirk and Taylor, 1957), one should also expect BMI to be a good predictor of relative Vo_2max and thus of the risk of EHI. However, if BMI were related to individual EHI risk <u>only</u> as a predictor of Vo_2max, then BMI and PFT run time should not be independent predictors of EHI risk. The foregoing observations thus raise the possibility that obesity may increase individual risk of EHI through some mechanism other than an effect on the regulation of T_{core}.

The purposes of the work described in this report are:

(a) to characterize certain normal physiological, hematological, biochemical, and immunological responses to

exercise-heat stress during basic training of Marine Corps recruits. The characterization will be based on measurements made on recruits who did not experience an episode of EHI associated with the event in which the measurements were obtained.

(b) to compare these normal responses in male recruits with those in female recruits, and to compare these normal responses with the corresponding responses in recruits presenting with an episode of EHI.

(c) to compare clinical features of EHI in male and female recruits.

Data collection and measurements performed during this reporting period include continued collection of clinical records on EHI patients, and measurement of responses of normal recruits during training events that are associated with high incidences of exertional heat illness, i.e., middle distance (2-4 miles) runs and the "Crucible", a 52-hour exercise, new this year (see below). Responses measured in normal recruits included core temperatures and levels of certain hematological and serum chemistry values that are part of the standard work-up of recruits with suspected EHI. In addition, blood samples were collected for later measurement of levels of gram-negative endotoxin and several cytokines, and for harvest of messenger RNA from white blood cells. The background for these measurements will be treated separately in the discussion that follows.

Blood and Body Core Temperature Measurements

Historically, heat exhaustion and heatstroke have often been thought of as two entirely distinct clinical entities, of which

heat exhaustion has been considered to be purely a disturbance of cardiovascular homeostasis, caused by impairment of venous return secondary to dehydration and peripheral pooling of venous blood, while heatstroke has been thought to be the result of a direct injurious effect of high temperatures on tissue. Many cases of heat exhaustion probably do represent relatively pure disturbance of cardiovascular homeostasis; however, other cases include evidence of cellular injury, such as elevated serum levels of various enzymes (Hubbard and Armstrong, 1988; cf. Table 1, based on recent experience at Parris Island). Moreover, there is no clear demarcation between heat exhaustion and heatstroke in practice, and many cases of heat illness could equally plausibly be classified either as severe heat exhaustion or as mild or early heatstroke. (Exertional heat injury, which appears in more recent literature on heat illness, may be a preferable diagnosis in many such cases.) It is likely, as Costrini et al. (Costrini, Pitt, Gustafson, and Uddin, 1979) and Hubbard (1979) have hypothesized, that heatstroke, exertional heat injury, and heat exhaustion (or at least those cases of heat exhaustion that involve more than a simple disturbance of cardiovascular homeostasis) represent parts of a continuum, sharing many of the same injurious processes.

Evidence has existed for some time that tissue temperature is not the only factor involved in the pathogenesis of heat illness. Hubbard (1979) analyzed exertional and non-exertional (classical) heatstroke, both in human patients and in an experimental rat model, and found that heatstroke may be produced by a less intense or shorter elevation of body core temperature in combination with heavy exercise, than in the absence of exercise.

He therefore concluded that some non-thermal factor or factors related to exercise increase susceptibility to heatstroke. In addition, analysis of data on exertional heat illness at Parris Island during the years 1982-1991 showed a strong association between daily incidence of heat casualties and maximum wet-bulb globe temperature the day before occurrence, suggesting the operation of some sort of cumulative effect of heat stress—perhaps accumulation of some harmful substance or depletion of some beneficial substance.

Blood Measurements. It has not been demonstrated what the non-thermal factors involved in heat illness are, but published data point to certain promising candidates, in particular LPS (Bouchama, Parhar, El-Yazigi, Sheth, and Al-Sedairy,1991; Butkow, Mitchell, Laburn, and Kenedi, 1984; Bynum, Brown, DuBose, Marsill, Leav, Pistole, Hamlet, LeMaire, and Caleb, 1978; Fine, 1975; Gathiram, Wells, Brock-Utne, and S. L. Gaffin, 1987; Graber, Reinhold, Breman, Harley, and Hennigar, 1971) and several cytokines (Bouchama et al, 1991; Bouchama, Al-Sedairy, Siddiqui, Shail, and Rezeig, 1993). Evidence for the involvement of these substances consists in demonstrations of their presence in blood and tissues of heatstroke patients and animals with experimentally-induced heatstroke; successful protection of experimental animals against heatstroke by pretreatment with broad-spectrum antibiotics to reduce the intestinal flora, or with Anti-LPS immune serum; and the consistency of the effects of these substances with many of the clinical manifestations of heat illness. The source of the LPS is the gram-negative bacteria in the normal intestinal flora; and although this LPS is normally confined quite effectively within the intestines, certain

stresses—including exercise (Bosenberg, Brock-Utne, Gaffin, Wells, and Blake,1988), heat (Gathiram, Gaffin, Brock-Utne, and Wells, 1987; Gathiram, Wells, Raidoo, Brock-Utne, and Gaffin,1988), and hypoxia (Gaffin, Brock-Utne, Zanotti, and Wells, 1986)—cause appearance of LPS in the systemic circulation. Since endotoxemia follows temporary intestinal ischemia produced by clamping the superior mesenteric artery (Gathiram, Gaffin, Wells, and Brock-Utne, 1986), it is likely that endotoxemia during exercise, heat stress, or hypoxia is a consequence of splanchnic vasoconstriction, which occurs as a cardiovascular homeostatic reflex (Rowell, 1974, 1983). It is likely that dehydration, by causing hypovolemia and compensatory splanchnic vasoconstriction, aggravates the endotoxemia associated with exercise and heat exposure, although we know of no study in which this hypothesis has been tested. In addition dehydration (Morimoto, Murakami, Ono, and Watanabe, 1986), muscle injury due to overexertion (Cannon, Medyani, Fielding, Fiatarone, Medyani, Farhangmehr, Orencole, Blumberg, and Evans, 1991), and perhaps other factors related to exercise and heat stress magnify the cytokine response to LPS. One possible way in which such factors might magnify the cytokine response to a later exposure to LPS is by inducing the transcription of messenger RNA for the respective cytokines. For this reason, we are taking aliquots from blood samples drawn from healthy control recruits, and treating the aliquots for later harvest of RNA.

We are aware of only three published studies that report levels of LPS or cytokines in the blood of human heat-illness patients, and all three compare levels in patients to those in normal controls who have not recently undergone substantial

exercise-heat stress. One of these (Graber et al., 1971) is a report of a single case of exertional heatstroke, and the other two studies (Bouchama et al., 1991, 1993) are of patients who, during the 1989 and 1990 pilgrimages to Mecca, contracted a mixed form of heatstroke (i.e., heatstroke which combined features of the classical and exertional forms, and which was associated with levels of exertion milder than those ordinarily associated with exertional heatstroke). The patients in the latter two studies had admission levels of LPS, TNF-α, IL-1α, IL-1β, IL-6, and interferon-γ that were significantly elevated compared to those of normal controls (Bouchama et al., 1991, 1993). The interpretation of these results in terms of the pathogenesis of heatstroke is not straightforward, since as noted above exercise, heat stress, and dehydration cause appearance of LPS in the systemic circulation, and since LPS, and perhaps intense exercise itself, elicit cytokine secretion, so that elevated amounts of these substances in patients' blood (compared to normal controls), may simply indicate recent exercise, heat stress, and dehydration, rather than a role for these substances in the pathogenesis of the patients' illness. Likewise, overexertion may cause skeletal muscle injury which is reflected in various serum enzyme levels and other chemistry measurements. However, the acute effect of basic training events on these measurements is not known, so that in attempting to account for the characteristic changes in hematological and clinical chemistry values that are associated with EHI, it is not possible to say to what extent these changes simply reflect recent intense exercise-heat stress, and to what extent they represent part of the pathogenesis of EHI. Healthy recruits training under similar

conditions probably experience a very similar type and degree of exercise-heat stress to that experienced by the patient. Therefore measurements gotten on such recruits, under conditions as close as possible to those associated with the onset of the patient's heat illness, should be the best possible control measurements for interpreting corresponding measurements made on the patient, and for drawing inferences about the significance of these measurements for the pathogenesis of EHI.

If LPS and cytokines do have a role in the pathogenesis of EHI, one should expect Anti-LPS and naturally-occurring antagonists of cytokine action to protect against EHI, and perhaps to contribute to improved heat tolerance following heat acclimatization and physical training. High levels of Anti-LPS do appear to improve heat tolerance (Brock-Utne, Gaffin, Wells, Gathiram, Sohar, James, Morrell, and Norman, 1988), and may be increased by aerobic training (Bosenberg et al,1988). In addition, Anti-LPS (Gaffin,1988; Gaffin, Gathiram, Wells, and Brock-Utne,1986; Pudifin, Lhoste, and Gaffin,1985; Wells, Gaffin, Gregory, and Coovadia, 1987) and a naturally-occurring antagonist of IL-1 (Ohlsson, Björk, Bergenfeldt, Hageman, and Thompson, 1990) are therapeutically beneficial in gram-negative shock.

Core Temperature Measurements. The quantitative relation between core temperature and risk of occurrence of heat illness is not well understood. Part of the reason is that under conditions in which heat illness is likely to occur, core temperature ordinarily is not measured in those who do not become sick. Moreover, as noted above, elevated core temperature is not the only factor involved in the pathogenesis of heat illness, so

that core temperature by itself may be of limited value in predicting the likelihood of occurrence of heat illness. At any rate, there presently is no reliable basis for predicting the risk of heat illness based on level of core temperature, nor are there sufficient data to determine how valid a predictor core temperature, by itself, is. Dr. Richard Gonzalez, Chief of the Biophysics and Biomedical Modeling Division of USARIEM, has confirmed that the estimates of heat casualty rates in the USARIEM Heat Strain Model, which are computed from predicted core temperature, are based on anecdotal information and informed guesses.

Some authors have noted that high core temperatures during exercise are better tolerated by some individuals than by others, and under some conditions than under others. [Sawka, Young, Latzka, Neufer, Quigley, and Pandolf (1992) include a brief general discussion of the problem, and Montain, Sawka, Cadarette, Quigley, and McKay (1994) address the effect of clothing during exercise-heat stress in conditions where the rate of heat production exceeds the maximum possible rate of heat dissipation.] However, the effects of factors that alter tolerance to high core temperatures have not been well defined; and because of the scarcity of core temperature measurements in healthy persons under conditions in which heat illness is likely to occur, it is not known to what extent those who actually become heat casualties do so because they have higher core temperatures than those who stay well, and to what extent they become heat casualties because they tolerate high core temperatures less well. As a corollary, therefore, the potential

for improving tolerance to heat stress by means other than improving thermoregulation is not well understood.

Differences Between Men and Women in Responses to Exercise-heat Stress and Clinical Presentation of EHI

Little is known about women's susceptibility to EHI. The literature on EHI in women is extremely sparse, and exertional heatstroke in women is considered to be rare (Knochel, 1989). It is not known whether the apparent rarity of exertional heatstroke in women owes to their being less frequently exposed to situations in which exertional heatstroke is likely, to behavioral factors (e.g., a lesser willingness to push themselves beyond safe limits), or to biological differences in tolerance to heat stress. The best available information about heat tolerance of women is based on laboratory studies on small numbers of subjects [See Kolka (1992) for a recent review.] Women have been reported to be less tolerant than men to passive heating and to exercise-heat stress. However, when male and female subjects are matched for individual factors that are known to affect heat tolerance, such as anthropometric characteristics and maximal O_2 uptake, there is little difference in thermoregulatory responses during short-term (< 45 min) exercise-heat stress, at least when the women are in the follicular phase of the menstrual cycles (Stephenson and Kolka, 1988). During the luteal phase of the menstrual cycle, the thermoregulatory setpoint is shifted upward about 0.5°C, but otherwise the phase of the menstrual cycle seems to have little effect on thermoregulatory responses and heat tolerance. However, during exercise of longer duration (> 60 min), heart rate and core temperature are higher than those

prevailing at apparent steady state at the end of shorter periods of exercise at the same intensity. (The higher heart rate is part of a phenomenon known as "cardiovascular drift".) It is probably in association with such prolonged exercise-heat stress that EHI is most likely to develop, but little is known of the responses of women to such prolonged exercise-heat stress.

We are unaware of any published comparisons of men and women with respect to susceptibility to EHI or clinical presentation of EHI. However if, as recent work suggests, inflammatory/ immunological processes are important in the pathogenesis of EHI, there may be important differences between men and women in heat tolerance and susceptibility to EHI, since reproductive hormones and the menstrual cycle are known to affect these processes. Data from our ongoing epidemiological study of EHI at Parris Island MCRD suggest that this may in fact be the case, since these data show differences between male and female recruits in the clinical presentation of EHI.

At Parris Island, our data show that male recruits and female recruits are almost equally likely to experience EHI (10% of recruits and 11% of EHI patients are female). However, 11% of male EHI patients are hospitalized, while female EHI patients are almost never hospitalized (Table 2): the only female EHI patient hospitalized during the years 1980-1995 had a co-existing sickle-cell crisis. The difference between male and female recruits in rates of hospitalization appears not to represent simply a difference in severity, but to be part of a more complex difference in the clinical presentation of EHI. In cases occurring in 1990-1991, mean peak rectal temperature was 101.6°F in female patients (all non-hospitalized) and 102.7°F in non-

hospitalized male patients; but mean peak serum levels of enzyme indicating cellular damage were all higher in females: CPK of 2215 vs 1571, AST of 71 vs 55, and LDH of 322 vs 297, suggesting that in women with EHI, a given level of rectal temperature may be associated with a more serious degree of tissue injury than in men, and that there may be quantitative differences in the pathogenesis between men and women. If so, it is likely that some features of EHI in female recruits vary with phase of the menstrual cycle. The Branch Medical Clinic at Parris Island began in 1993 to record date of last menstrual period for all female heat casualties, but the number of EHI cases in female recruits since then is not yet sufficient to show any pattern in those data.

BODY

OVERVIEW OF THIS REPORTING PERIOD

Until mid-August, weather during the 1997 hot season was milder than usual, and the number of EHI cases for the season was correspondingly low. Approximately 100 EHI cases came through the Branch Medical Clinic, and of these approximately 45 were cooled in the Cold Room in the clinic. Since the 1996 hot season, two changes in the recruit training program were made that have had, or are expected to have, important effects on the epidemiology of EHI. One of these changes is the introduction of the "Crucible" into the eleventh (next to last) week of training. The Crucible is a 52-hour field exercise which begins and ends with a 9-mile march with pack and weapon, and lasts from 0400 Thursday morning to 0800 Saturday morning. During the Crucible,

recruits get about 4 h of sleep a night and are issued limited rations, though drinking water and sugar-electrolyte drink are freely available. Even though the Crucible does not involve significant amounts of running, it accounted for an estimated 40% of EHI cases during the 1997 hot season. Most of the heat casualties during the Crucible occurred during the final 9-mile march. This is in sharp contrast to previous years, when most EHI cases occurred during running, and more than half occurred during the first four weeks of training. The other change in the recruit training program is an increase in the distances that female recruits run, in order to enable them to meet the same physical fitness standards for running that are required of the males. We expect this change to increase the incidence of EHI in female recruits with respect to that in male recruits, but we have not yet analyzed the data for the 1997 hot season to determine whether this has, in fact, happened.

METHODS

Volunteers

We enroll approximately equal numbers of normal volunteers of each sex from Marine recruits undergoing basic training at Parris Island MCRD. During the first reporting period we administered swallowed temperature-sensing telemetry devices to approximately 1300 volunteers; collected blood samples from 30 female and 35 male controls and 4 EHI patients to measure several cytokines and indices of leukocyte function; and assessed cognitive function of 277 control recruits and 14 recruits who experienced a clinically significant episode of dilutional hyponatremia. During this reporting period we enrolled and

tested two groups of normal recruits. The first group included 14 men and 6 women going through the crucible. Volunteers in this group completed a questionnaire on enrollment and on each morning of the Crucible; provided seven blood samples, i.e., at 0500 and 1700 hours on the day before and on the first two days of the Crucible, and at the end of the Crucible; and had their core temperatures monitored from 0500 to 1700 hours on the first two days of the crucible and during the final march. The second group includes 80 volunteers, i.e., a cohort of 20 volunteers from each of four training companies, two male and two female. Each cohort was tested during four events: middle-distance runs (2.5 to 4 miles) at three stages (early, middle and late) during training and during the final march of the Crucible. These volunteers completed a questionnaire on enrollment and on the morning each test day; provided blood samples during in-processing (during their first week at Parris Island, before beginning training), at the beginning and end of each run or march, and after recovery from the run or march; and had their core temperatures monitored during the run or march. The blood samples included tubes sent to the clinical laboratories at Beaufort Naval Hospital for the hematological and serum chemistry analyses that are part of the standard work-up of recruits with suspected EHI, and tubes that were processed for later measurement of levels of gram-negative endotoxin and several cytokines, and for harvest of messenger RNA from white blood cells. We intend to test one male and one female cohort in the same manner during winter 1998 and spring 1998, and two male and two female cohorts during summer 1998.

Experimental Design

Questionnaires. On enrollment in the study, each volunteer completed a background questionnaire, asking about factors that may account for individual differences in core-temperature responses to exercise, including previous occupation and physical activity level, medical history, current medications, and recent consumption of nutritional supplements. On the morning of each event, each volunteer completed another questionnaire asking about current psychological state, watches and sleep the previous night, fluid consumption during the previous 24 hours, medical history during the previous two weeks and, for each female recruit, date of her last menstrual period.

Collection of blood samples. Each blood sample consisted of two 7-ml glass Vacutainer® tubes (purple-topped) and one 10-ml polypropylene tube with EDTA as an anticoagulent, and a 10-ml red-topped Vacutainer® tube (no anticoagulent). On each occasion, the red-topped and one purple-topped Vacutainer® tube were sent to the clinical laboratories at Beaufort Naval Hospital for the measurements that are part of the standard work-up of recruits with suspected EHI—i.e., hematocrit, hemoglobin, white cell count, differential, electrolytes, glucose, BUN, creatinine, uric acid, AST, ALT, LDH, and CPK. (AST, LDH, and CPK are elevated with muscle injury, and AST, ALT, and LDH are elevated with liver injury.) The other purple-topped Vacutainer® tube was put into melting crushed ice until it was centrifuged, and the plasma was decanted sterilely and frozen for later measurement of gram-negative endotoxin. The final tube in each set was collected for measurement of several cytokines and harvest of messenger RNA from white blood cells. This tube was made of polypropylene since

glass activates white cells and would affect these analyses. Aliquots of whole blood from each tube were incubated with tri-reagent and then frozen for later harvest of RNA. The rest of the blood was centrifuged and aliquots of the supernatant plasma were removed and frozen to provide a sample for measurement of cytokines and a spare plasma sample. All samples were kept frozen on Dry Ice and sent for later analysis to USARIEM.

Continuous recording of body core temperature. We measured body core temperature with ingested CorTemp® (H.I. Technologies, Inc., St. Petersburg, FL) telemetric temperature sensors. The temperature signal is transmitted via an antenna worn on the volunteer's belt in front to a small data recorder worn on the belt in back. While the sensor is in the stomach, it is affected by the temperature of anything that the volunteer ingests (e.g., a drink of cold water). Therefore sensors were administered between 8:00 p.m. and lights out the evening before we intended to collect data, so that they would be in the small intestine when data collection began.

We chose the training days on which to record core temperature and collect blood so as to provide a good representation of those training events associated with high rates of EHI (chiefly distance runs, but including also the Crucible), and also so as to allow comparison of similar events that are repeated at different stages of training. We recorded temperature continuously for the duration of the day's chief physical training session, approximately 3 hours except during the Crucible, when data collection lasted up to 12 hours at a stretch.

Collection and organization of clinical data on EHI patients. For all identified EHI episodes during 1997

(approximately 100 cases), we made photocopies of pertinent clinical and narrative records. *These include all relevant pages in the patient charts (including follow-up clinic visits) that pertain to these cases, all clinical laboratory reports, and all pertinent Recruit Incident Reports (RIBS), which are filed by the Drill Instructors. The Branch Medical Clinic uses standard clinical data forms, and obtains a standard panel of laboratory evaluation, for suspected EHI patients. These forms and the standard panel were originally designed for an epidemiological study of EHI, and continue in use, with some modifications, for the present study. These forms are structured so as to record circumstances of the onset of the episode of EHI and clinical information including treatment and serial vital signs and neurological evaluation, in a standard format.* Weather data are obtained from hourly measurements of WBGT made at Parris Island MCRD and the nearby Marine Corps Air Station (MACS) at Beaufort, SC during the hot season, and also from standard meteorological data recorded at the MCAS. The training activity at the onset of EHI episode is obtained from the clinical data forms and the RIRs, and confirmed using the applicable published recruit training schedules. However, some EHI cases are identified only in retrospect, and it is often necessary to retrieve some of the pertinent clinical records from the patient's next duty station.

When this report was prepared, all EHI cases through 1996 had been reviewed and entered into the computerized database.

Specific measurements and techniques. Analyses of the blood samples obtained from EHI patients and healthy controls will be carried out by J. S. Kennedy, MAJ, MC or his technician in MAJ Kennedy's laboratory at USARIEM. Plasma in these samples will be

assayed for inflammatory cytokines interleukin (IL)-1β, tumor necrosis factor (TNF), and IL-6. The anti-inflammatory cytokines IL-1 receptor antagonist (IL-1Ra) and soluble cytokine and cell adhesion receptors will be assayed from plasma to assess the balance between pro-inflammatory and anti-inflammatory host response during heat illness. All analyses on plasma will be performed using commercial kits. To elucidate mechanisms by which host inflammatory response to heat illness is modulated, we will harvest messenger RNA from peripheral leukocytes (contained in the buffy coat) and analyze for gene products that regulate the inflammatory response. By use of reverse transcription-polymerase chain reaction we will determine mRNA levels for IL-1, IL-2, γ-interferon, and markers of gene regulation such as c-for, c-jun, and AP-1.

RESULTS

Continuous recording of body core temperature.

Although we administered over 1600 CorTemp sensors to our volunteers, we have only approximately 500 usable temperature records from male recruits, and 450 from female recruits. (About 60 of these records include data obtained during road marches with loads, and the rest include data obtained during distance runs.) Inspection of plots of T_{core} against time shows that in the hot season, the recruits never reach thermal steady state during distance runs, and T_{core} continues to rise until the end of the run. A contract is in place to analyze the core-temperature records to generate equations that will predict an individual's T_{core} during a run as a function of time, WBGT, the individual's

BMI and PFT run time, week of basic training, sex and, for female recruits, phase of the menstrual cycle.

Problems. As noted in the previous paragraph, a high proportion of records of core temperature were not usable. We believe that several identified technical reasons account for the unusable core temperature records. These include the directional nature of the signal from the temperature sensor, which causes the strength of the received signal to vary according to the sensor's orientation with respect to the antenna; feed-back interference between the receiver/recorder and antenna; and high current demands of the present (first-generation) receiver/ recorder, which sometimes runs the batteries down before the end of a test. A newer-generation receiver/recorder is soon to go into production, and some prototypes have been field tested; and the newer-generation receiver/recorder should obviate some of these problems. It requires a much lower current so that one battery will last for several weeks of operation, rather than several hours; it has three internal antennas in three orientations, to maximize the strength of the received signal; and there appears to be no problem due to feed-back interference. CDR Gastaldo has ordered several of the newer-generation units with Navy funds.

As a safeguard against using defective or wrongly-calibrated temperature sensors, we continue to check the accuracy and stability of the calibration of each sensor using a temperature-controlled water bath and certified mercury-in-glass thermometer as described in the previous progress report. In addition, we have gotten the manufacturer to increase the number of significant digits in the slope and intercept of their furnished calibrations

from three to four, and on receipt of each shipment, temporarily turn each sensor "on" to check the strength of the signal.

Analysis of blood samples.

Only the clinical hematological and chemistry analyses have been performed so far on blood collected during this reporting period. The most noticeable changes have been in samples collected during the Crucible, in which we frequently observed a slight downward trend in serum [Na^+] (due presumably to drinking large volumes of fluid, as the subjects report in the questionnaires) over the course of the exercise, and a fairly consistent gradual increase in serum levels enzymes (AST, LDH, and especially CPK) associated with muscle injury. In several cases, final levels of CPK were greater than 3000 IU/L (vs. laboratory normal values of 22-269 IU/L.) These changes are interesting because of the prominence of elevated levels of these enzymes in the clinical presentation of EHI, and because of the possible association between muscle injury and stimulation of inflammatory responses (Cannon, Medyani, Fielding, Fiatarone, Medyani, Farhangmehr, Orencole, Blumberg, and Evans, 1991). When the immunological assays are performed on the corresponding samples, the results will be compared to these enzyme levels.

In the previous progress report it was reported that we collected blood samples for immunological measurements from four EHI patients and 65 controls. These samples were analyzed only for IL-6, since levels of that cytokine remain elevated longer than those of the others that we intend to measure, and we thought it the most likely to show elevated levels. We did not find any elevated levels, but the reagents used for those analyses were old

and may have lost their potency. We plan to repeat them with fresh reagents.

CONCLUSIONS

Collection of blood samples.

The requirements for collecting and handling blood for our intended immunological assays are rather demanding, especially in the case of the specimens to be analyzed for LPS and those from which we plan to harvest RNA. During the coming winter we expect to analyze and harvest RNA from a representative sample of the blood collected during the past few months. The quality of these specimens will provide a test of how well we have the necessary procedures in hand. If, as we hope, these samples prove to be of good quality, they will show that we do have the necessary procedures well in hand. If not, we will need to review our procedures, make whatever changes are indicated, and collect and analyze another test batch of samples before carrying out large-scale collections next year.

Continuous recording of body core temperature.

The equations that we expect to generate predicting an individual's T_{core} during a run as a function of time, WBGT, the individual's BMI and PFT run time, week of basic training, sex and, for female recruits, phase of the menstrual cycle, will furnish norms against which to compare presenting rectal temperatures of EHI patients. We will thus have a basis for drawing conclusions regarding the extent to which development of EHI is associated with patients' reaching a higher level of T_{core}

than levels reached by healthy recruits performing the same activities under the same conditions, and the extent to which it is associated with patients' being less tolerant to high T_{core}. Furthermore, we will have a basis for inferences about whether individual risk factors (e.g., high BMI) for developing EHI act because of an association with high levels of T_{core} during exercise or in some other way.

Collection and organization of clinical data on EHI patients.

Introduction of the Crucible into the recruit training program this year allows us to study both normal responses and EHI occurring during more moderate but sustained activity that middle-distance running. For this reason, the Crucible may thus provide a better model than running for EHI occurring during operations. Analysis of the EHI cases occurring during this year's Crucible will allow us to explore this notion further, and may allow us to plan studies of normal recruits during the Crucible to better advantage.

In addition, as a result of changes in the training program for female recruits, the amount of running that they do is now more similar to that of the men than it was in past years. Moreover, a large fraction of this year's EHI cases occurred during the Crucible, in which exercise by the women is similar to exercise by the men. Both of these factors should reduce the impact on EHI of training differences between men and women. Therefore analysis of the data on this year's EHI cases should help us to assess more critically the apparent gender differences in presentation and severity of EHI.

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APPENDIX A

ABBREVIATIONS

ALT(SGPT) – alanine aminotransferase

ANAM – Automated Neuropsychological Assessment Metrics Battery

Anti-LPS – antibody against LPS

AST(SGOT) – aspartate aminotransferase

BMI – body mass index (weight/height^2)

CPK – creatine phosphokinase

EDTA - ethylene diamine tetra-acetic acid, used as an anticoagulant

EHI - exertional heat injury

ELISA – Enzyme-linked immunosorbent assay

IgG – immunoglobulin G

IL-1, IL-1α, IL-1β, IL-6 – interleukin 1, interleukin 1α, interleukin 1β, interleukin 6

LDH – lactic dehydrogenase

LPS – lipopolysaccharide endotoxin

MCRD – Marine Corps Recruit Depot

PFT – Physical fitness test

T_{core} – body core temperature

TNF – tumor necrosis factor

USARIEM – US Army Research Institute of Environmental Medicine

$\dot{V}o_2max$ – maximal rate of O_2 uptake